

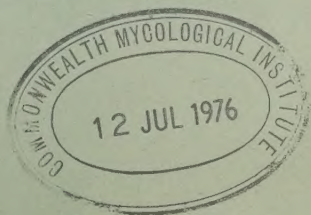
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FURTHER STUDIES OF THE BROWN-ROT FUNGI.

V. BROWN-ROT BLOSSOM WILT OF
PEAR TREES.



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Further Studies of the Brown-rot Fungi.

V. Brown-rot Blossom Wilt of Pear Trees.

BY

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With Plates **XLI** and **XLII**.

INTRODUCTION.

PRIOR to the publication, in 1900, of Woronin's (10) paper on the fruit-rotting *Sclerotinias*, it was generally considered that the brown-rot diseases of fruit trees were caused by one species of fungus, *Monilia fructigena*, Pers. = *Sclerotinia fructigena* (Pers.) Schröter. Woronin showed clearly that in Europe two fungi were responsible for these diseases, and that *Sclerotinia cinerea* (Bon.) Schröt. was quite distinct from *S. fructigena* proper, in the morphology of its conidial fructifications and in its relation to the host plants. The ascigerous fructifications of these fungi had not, at that time, been recorded.

According to Woronin *S. cinerea* was confined almost exclusively to the stone fruits, whilst *S. fructigena* infected chiefly the core fruits. More recent observations have shown, however, that the host relations of these species are not so restricted as was formerly thought. Thus in 1917 a serious blossom wilt of apple trees in Britain, caused by a form of *S. cinerea*, was described (6). Moreover in recent years *S. fructigena* has been responsible for considerable rotting of the stone fruits, and in some instances the fruit rot of plums caused by this fungus has far exceeded that produced by *S. cinerea*.

It is true that on apples and pears brown rot of the fruit is caused almost solely by *S. fructigena*; on these hosts *S. cinerea* is rarely associated with fruit rot. *S. fructigena* is not known to cause natural infection of flowers and is therefore not associated with blossom wilts. *S. cinerea*, on the other hand, not only infects the fruit of *Prunus* spp., but also infects flowers and causes blossom wilt of plum, cherry (particularly the Morello cherry), apple, and, as shown below, of pear trees.

That *S. cinerea* is able to infect pear trees appears to have been observed first by Jackson, who, in 1915, published a short description of a pear canker in Oregon (5). The fungus associated with this disease was given the name *Monilia oregonensis*, Barss and Posey, but it has since been shown that this fungus is indistinguishable from *Sclerotinia cinerea* (Bon.) Schröter (9).

The only description that I have been able to find of a similar disease on pear trees in Europe, is a brief note which appeared in 1928 mentioning *Monilia fructigena* as infecting flowers of apple and pear in Italy (1); the colour of the fungus, however, is given as grey ('grigio'), so that probably the fungus was *M. cinerea*. It should be mentioned, too, that Eriksson (4), in describing a brown-rot blossom wilt of apples in Sweden, states that a grower informed him of a similar disease on pears, but no details are given.

SCLEROTINIA CINEREA ON PEAR TREES IN ENGLAND.

The association of *S. cinerea* with diseases on pear trees in England first came under my own observations in 1915 on finding, on a pear tree in a plantation in East Kent, a young fruit, about half an inch long, bearing small grey pustules typical of the *Monilia* stage of *S. cinerea*.

A similar small pear fruit bearing fructifications of *Monilia cinerea* was sent to me in 1921 by Mr. A. D. Cotton, who had received the specimen from Exeter. The dimensions of the conidia on this pear showed variation from 11.5×8 to $28 \times 20 \mu$ with an average of $18 \times 12.5 \mu$. These dimensions are of the same order as those of the conidia of *S. cinerea* as found on infected plums in summer.

A blossom wilt of pears associated with the presence of *S. cinerea* on the flowers was brought to my notice in 1920, also by Mr. Cotton,¹ who sent me a truss of withered flowers and leaves from Cambridgeshire; the variety of pear was not ascertained. Small grey *Monilia* pustules were present on the flowers (calyx tube and pedicels) and on the axis of the inflorescence. The conidia measured 9×6.5 to $19.5 \times 13 \mu$, average $16.5 \times 9.5 \mu$. The mode of germination of these conidia on prune agar plates, and the form of the mycelial growth were similar to those of *S. cinerea* found on plums.

In June 1921 young pear trees of the variety Fertility, growing in the plantation of the South-Eastern Agricultural College, Wye, were seen to be infested with blossom wilt. One tree bore 22 wilted inflorescences, or about 4 per cent. of the total number. All the flowers and leaves on each infected spur were withered, but the infection did not extend further than the base of the spur itself, so that there were no cankers on the branches.

¹ The author takes this opportunity of acknowledging Mr. Cotton's help in providing specimens.

Monilia pustules were present on some of the flowers. Conidia taken from one of these pustules measured 9×8 to 24×12 and $17.5 \times 15 \mu$ with an average of $16 \times 11 \mu$.

On attempting to obtain cultures by isolating conidia on culture plates, there was found to be so much contamination from bacteria that that method was abandoned and plates were prepared by sterilizing the outer surface of an infected spur and then transferring particles of the internal tissues to prune agar plates. From these particles hyphae grew out and produced cultures which were, apparently, quite pure. Such cultures on prune agar did not produce spores, but inoculations from them on to sterilized potato resulted in a good crop of conidia within seven days, and from these conidia monospore cultures were obtained.

During the winter of 1921-2 the trees were subjected to the usual routine pruning, but when they were examined in February 1922 a number of the spurs, which had been overlooked during the pruning operations, remained, and these bore grey *Monilia* pustules. The conidia on these pustules were much smaller than those found on the flowers the previous season. Their dimensions were 8.5×6 to 14×10 and $15 \times 9.5 \mu$, with an average of $11.5 \times 8 \mu$. These dimensions, again, are in conformity with those recorded for *S. cinerea* f. *pruni* on plums and for *S. cinerea* f. *mali* on apple spurs, when produced on pustules which develop during the winter (8). Monospore cultures were obtained by isolating conidia taken from these winter pustules on a dead pear spur, and the resulting cultures were similar to those obtained from the tissues of a spur in the previous June. In May, 1922, a few infected inflorescences were found on two of the trees, the infection, it must be assumed, being caused by conidia produced on those spurs which had been killed the previous year and not removed from the trees.

In 1925 Mr. J. Amos collected specimens of pear blossom wilt from Fertility pear trees at Wisborough Green, Sussex. He estimated that there were some seventy or eighty infected trusses per tree on trees six to seven feet high. One of these specimens showed a dead terminal spur (result of infection in 1924) bearing pustules, and a few inches below it were two wilted inflorescences recently infected, also bearing pustules (see Pl. XLII, Fig. 4). The fungal fructifications of the old spur were on the flowers, pedicels, petioles, and the axis of the spur; on the current year's inflorescences the pustules were on the calyx tubes and pedicels only.

Field observations have shown, then, that Fertility pear trees are subject to a blossom wilt associated with the appearance of the *Monilia* stage of *S. cinerea* on the dead flowers and spurs. As has been noticed in other cases of infection by this species the conidia produced on the spurs in the winter and early spring are noticeably smaller than those which develop on the newly infected flowers during the summer. In culture the

fungus resembles *S. cinerea* forma *pruni* rather than forma *mali* in that the dark coloration, characteristic of the latter in prune agar cultures, was lacking, and also that on sterilized potato there was copious development of conidia, not scanty as in the apple blossom wilt fungus.

The constant association of *S. cinerea* with this form of blossom wilt in pears, and the fact that forms of this fungus are known parasites on other kinds of fruit trees were good presumptive evidence that the fungus was the cause of the wilt, but experimental proof was required for confirmation, particularly as a bacterial disease of pears, described by Barker and Grove (2), produces somewhat similar symptoms. Again, since the fungus is morphologically and culturally similar to *S. cinerea* f. *pruni* it was necessary to determine whether *S. cinerea* as found on plums and cherries is able to infect pear flowers.

INOCULATION EXPERIMENTS.

(A) *Inoculation of Pear Flowers.*

Attempts to prove experimentally that the fungus is the cause of the blossom wilt were at first unsuccessful. All inoculations of flowers on Fertility pear trees in the open, using conidia of strains from naturally infected pear inflorescences, have given negative results. Such inoculations have been carried out in the field during four different seasons, but in no case did blossom wilt occur.

In 1924 greenhouse accommodation and suitable trees were available at the East Malling Research Station, so inoculations were carried out under glass. A fairly moist atmosphere was maintained by watering the floor of the greenhouse frequently, and the trees were occasionally sprayed with water. The inoculations were made with conidia taken from a pure culture of *S. cinerea* isolated from an infected pear spur, and grown on sterilized potato.

In order to ascertain what parts of the flower were most susceptible to infection, conidia were placed on (1) the disc, (2) petals, (3) receptacle, and (4) stigmas. The conidia were transferred from the culture to the organs to be inoculated by camel hair pencils which had previously been boiled for five minutes and allowed to cool. The pencils were used slightly moist so as to avoid the possible dispersal of the spores by currents of air. The results were as follows:

(1) *Inoculation of the floral disc.* Seventeen flowers inoculated. There was no definite evidence of infection, though in some flowers a slight discoloration of the disc was observed which was not to be seen in flowers not inoculated.

(2) *Inoculation of petals.* Two to five flowers on each of eight inflorescences were inoculated on the lowest two petals; the flowers were

first sprayed with sterilized distilled water, and conidia were then placed in the drops of water which collected on the concave inner surface of the lower petals. In all, 29 flowers (58 petals) were inoculated. Many of the inoculated petals showed a browning at the inoculated spots in two or three days, the spots in some cases being several centimetres in diameter, but, with one exception, all the inoculated petals fell off before the infection had extended into the base of the petals.

The one flower which eventually became infected was inoculated on two petals on April 30, the day the flower opened. Within two days each petal showed a brown blotch at the place of inoculation, and this had increased in size by the following day, when, however, one of the petals fell. The other inoculated petal remained attached, and on May 4 the browning had extended to the receptacle and to the two sepals nearest to the infected petals; the distal half of the petal was still white and unaffected. On May 5 the discoloration had extended into the pedicel, and by the next day had reached the base of the pedicel; the infected petal remained attached to the flower.

May 8. The whole axis of the inflorescence was discoloured; the leaves and the rest of the flowers were becoming blackened from the base upwards for 5 to 8 mm. and were flagging.

May 9. The whole truss was withered; conidial fructifications were present on some of the pedicels.

Although there was only this single example of definite infection there is good evidence that it was the result of inoculation, as shown by, (a) the discoloration of many of the inoculated petals before they fell, such browning not appearing on petals not inoculated, even on the same flower; (b) the progressive infection of the organs from the inoculated petal to the axis of the inflorescence; and (c) the appearance finally of *Monilia* fructifications on the flowers of the infected inflorescence.

(3) *Inoculation of the receptacle.* One to three flowers on each of four inflorescences were inoculated by making a puncture on the side of the receptacle and touching the spot with a brush bearing conidia. The inoculations were made on April 26, and control punctures were also made.

Although a slight discoloration was observed round some of the inoculated punctures there was no evidence of definite infection after ten days.

(4) *Inoculations of stigmas.* Six inflorescences were selected, and two, three, or four flowers on each inflorescence were inoculated on the stigmas, 21 flowers in all.

All the inoculated flowers showed the early stages of infection. There was first a browning of the stigmas; this gradually became more noticeable and then the discoloration passed down the styles until these were completely brown. The discoloration of the floral disc followed.

On one inflorescence infection ceased at this stage. On the others, however, infection soon appeared in the receptacle, and passed along the pedicel into the axis of the inflorescence. This was followed by the wilting of all the flowers and leaves on the spur, the result being typical blossom wilt.

The rate of progress of the infection (as shown by the discoloration) was fairly uniform throughout, and in general the symptoms noted, in relation to the length of time after inoculation, were as follows :

No. of days after inoculation.	Result.
3	Stigmas brown.
6	Styles (one or more) brown to base.
7	Disc discoloured.
9	Receptacle and part of pedicel infected.
10	Pedicels discoloured to base.
11	Axis of inflorescence invaded : leaves and flowers flagging.
12	Leaves and flowers completely wilted.

It will be seen that the rate of progress was very slow during the first week. About the seventh day the infection had reached the disc at the base of the styles. After this progress was more rapid, probably as a result of the stimulus afforded by the foodstuffs in the nectar and in the tissues of the ovary. The pedicels of the flowers were 1.5 to 2 cm. long, and when the discoloration appeared at the distal end of a pedicel it extended towards the base at the rate of about 1 cm. per day, so that the axis of the inflorescence was then soon invaded.

In the infected flowers the calyx lobes and stamens remained turgid and healthy looking until the infection had reached the axis of the inflorescence, when the whole flower withered.

At the time that an inflorescence as a whole began to flag, the uninoculated flowers on it showed no discoloration except at the base, where they were being invaded from the axis; the petals by this time had fallen, but the styles, stamens, and sepals were turgid and quite normal in appearance.

Owing to the absence of pollination under the conditions of the experiment the styles of flowers not inoculated generally showed no discoloration at this stage, though a slight browning of the stigmas of some flowers was to be observed.

Later the tree was placed in the open. During the winter the infected spurs could be distinguished from the rest by the persisting withered flowers and leaf-stalks. Pustules of the *Monilia* stage of *S. cinerea* appeared on the withered organs during winter and spring (see Pl. XLII, Fig. 7).

In 1925 inoculations were again made on pear flowers in the greenhouse, using a pear strain of the fungus. The early symptoms of infection

were seen in the browning of the styles in most cases, but the flowers fell without any infection of the spurs. Probably in this experiment the air in the greenhouse was too dry for the infection to run its course.

In 1926 another series of experiments was carried out on pear flowers using a cherry strain, a plum strain, and, for comparison the pear strain used in previous experiments. Again conditions for infection were not wholly favourable, for although flowers on nine inflorescences were inoculated with each strain, and most of them showed definite evidence of infection, the discoloration extending into the receptacle and also, on some flowers, into the pedicel, only one spur was killed. The flowers of this inflorescence in which the infection was complete had been inoculated with the cherry strain.

A similar series in 1927 gave even less satisfactory results, most of the inoculated flowers showing merely infection of the styles; only two flowers (inoculated with a plum strain) showed infection of the disc and receptacle.

In 1928 a greenhouse more suitable for pathological experiments was available, and the inoculations were continued. Successful infections were obtained with a pear strain and also with a strain of *S. cinerea* isolated from a plum twig. With the latter five inflorescences were inoculated on two flowers each, and all the ten flowers became infected. The spurs were invaded and typical blossom wilt resulted. In a similar series of inoculations on five inflorescences, using a strain from a plum fruit, all the ten inoculated flowers became infected (producing at least a blackening of the disc), but only one spur was killed.

The trees on which these experiments had been carried out were placed in the open, where they remained over the winter. When they were examined in April, 1929, it was seen that some of the infected spurs bore *Monilia* fructifications which had developed during the winter and spring. Conidia taken from one of the spurs which had been inoculated with the pear strain were found to measure 8.5×6 to 12.5×10.5 and $14 \times 8.5 \mu$, with an average (100 conidia) of $10.5 \times 7.5 \mu$. It will be seen that these dimensions correspond to those of conidia obtained from naturally infected spurs during the winter months.

Convincing proof that a strain of *S. cinerea* isolated from a cherry can infect pear flowers was obtained in 1929. The inoculations were made on a young Fertility pear tree in the greenhouse. Ten inflorescences were inoculated on two flowers each. All the flowers became infected, in every case the discoloration extending to the base of the pedicel. On three inflorescences, however, the flower-bearing axis was not invaded. On one other the flower-bearing axis was blackened and killed, but the infection did not extend into the older part of the spur bearing leaves, and the latter remained alive and healthy. In the remaining six the spurs were killed to their base so that the flowers and leaves all wilted, the symptoms being characteristic of typical blossom wilt.

In this experiment it was observed on some spurs that when the discoloration had reached the base of one or both of the inoculated flowers and had entered the inflorescence axis, it then extended upwards along the pedicels and petioles before the whole truss wilted. On such leaves the discoloration was seen to travel along the midrib, and from the midrib laterally into the lamina.

(B) *Inoculations on Plums.*

Since the pear blossom wilt fungus resembled *S. cinerea* forma *pruni* morphologically and culturally, and since inoculation experiments with a plum strain had produced a blossom wilt of pears, it seemed certain that the pear fungus was identical with *S. cinerea* f. *pruni*. To obtain further confirmation of this two pear strains were used in inoculating plums (fruit) on trees in the open. The plums when inoculated were about half grown and quite green. A single puncture was made on each fruit with a sterile needle, and a drop of water, containing conidia from a pure culture, was placed on the puncture. Seven plums were inoculated with one strain and nine with the other, while eight were punctured without inoculation. All the inoculated plums showed definite evidence of infection within three days. On the fifth day one of them had a drop of gum only at the puncture, another showed infection extending about 5 mm. from the puncture, while the rest (fifteen in number) had each about half the surface discoloured and ten of them already bore the grey conidial tufts similar to those found on plums naturally infected with *S. cinerea*. There was no infection of the controls.

(C) *Inoculations on Apple Flowers.*

In 1924 a pear strain of *S. cinerea* was used in inoculations on apple flowers; similar inoculations were made at the same time with a strain of *S. cinerea* f. *mali* for comparison. Six inflorescences were inoculated on one or two flowers with the pear strain and five inflorescences with the apple strain.

All the inoculated flowers showed the early stages of infection with browning of the styles to the base, but only in two inflorescences, one inoculated with the pear strain and one inoculated with the apple strain, did complete infection occur with the typical wilting of flowers and leaves. Later these two spurs were cut off and were found to be invaded by mycelium. Isolations were made from each, and the fungus used in the inoculation recovered. The two strains could be distinguished from the fact that the pear strain was almost colourless on prune agar, whilst the apple strain produced a dark brown coloration. This distinction was retained after re-isolation from the apple spurs.

This experiment is only on a small scale and has not yet been repeated,

but the fact that the pear strain was re-isolated from the tissues of the spur of which the flowers had been inoculated with that strain is offered as evidence that, under the conditions of the experiment, the pear blossom fungus was able to infect the apple flowers.

In a series of experiments described in an earlier paper (7), inoculations on apple flowers with strains of *S. cinerea* from plum and cherry failed to produce typical blossom wilt of apple, and those results have recently been confirmed by Boyle, Murphy, and Cummins (3) in Ireland. It must be left an open question at present as to whether the pear strain is a form intermediate between *S. cinerea* forma *pruni* and *S. cinerea* f. *mali* with the cultural characters of the former but able to cause blossom wilt of apple, or whether *S. cinerea* f. *pruni* may under certain favourable conditions cause blossom wilt not only of pears but also of apples.

'SECTORING' IN THE PEAR BLOSSOM *MONILIA*.

One pear blossom strain, isolated in 1922 from a single conidium, remained apparently pure and unchanged until 1927, when a transfer to a prune agar plate gave rise to growth showing 'sectoring'. The abnormal form appearing in this culture has not yet been studied in detail, so that here it will be merely stated that its habit in plate cultures is different from the typical form, and when transferred to sterilized potato it produces conidia which are spherical, or nearly so, not definitely lemon-shaped as in the normal form. Inoculations with this 'saltant' on pear flowers have failed to produce blossom wilt, and inoculations on plums (fruit) have also given negative results.

SUMMARY.

1. A blossom wilt of Fertility pear trees is described.
 2. The fungus associated with the disease is morphologically and in culture indistinguishable from *Sclerotinia cinerea* forma *pruni*, and is therefore referred to that species.
 3. Inoculation experiments carried out with the fungus isolated from pear spurs have given rise to typical blossom wilt on pear trees.
 4. Inoculation experiments on pear flowers using strains of *S. cinerea* f. *pruni* isolated from plum and from cherry have also resulted in blossom wilt.
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LITERATURE CITED.

1. ANON: Cronaca dei parassiti e delle malattie. Curiamo le Piante, vi, no. 5, p. 99, 1928.
2. BARKER, B. T. P., and GROVE, O.: A Bacterial Disease of Fruit Blossoms. Ann. Appl. Biol., vol. i, pp. 85-97, 1914.
3. BOYLE, C., MURPHY, M., and CUMMINS, H. A.: 'Blossom-Wilt' of Apple Trees and 'Wither-Tip' of Plum Trees, with Special Reference to Two Biologic Forms of *Monilia cinerea*, Bon. Scien. Proc. Roy. Dublin Soc., vol. xix (N.S.), no. 8, pp. 63-76, 1928.
4. ERIKSSON, J.: Zur Kenntnis der durch *Monilia*-Pilze hervorgerufenen Blüten- und Zweig-dürre unserer Obstbäume. Mycologisches Centralblatt, ii, pp. 65-78, 1913.
5. JACKSON, H. S.: Pear Canker, *Monilia*, sp. Oregon Agric. Expt. Sta. Bienn., Crop and Pest Rept., 1913-14, ii, pp. 271-2, 1915.
6. WORMALD, H.: A Blossom Wilt and Canker of Apple Trees. Ann. Appl. Biol., vol. iii, pp. 159-204, 1917.
7. —————: The 'Brown-rot' Diseases of Fruit Trees, with Special Reference to Two Biologic Forms of *Monilia cinerea*, Bon. I. Ann. Bot., vol. xxxiii, pp. 361-404, 1919.
8. —————: II. Ibid., vol. xxxiv, pp. 143-71, 1920.
9. —————: A Contribution to Our Knowledge of the Distribution of the Species of *Sclerotinia* Causing Brown Rot. Ann. Bot., vol. xli, pp. 287-99, 1927.
10. WORONIN, M.: Über *Sclerotinia cinerea* und *S. fructigena*. Mém. Acad. Imp. Sci. St.-Petersbourg, viii^e sér., vol. x, no. 5, Phys.-Math., pp. 1-38, 1900.

EXPLANATION OF PLATES XLI AND XLII.

Illustrating Dr. H. Wormald's paper on Further Studies of the Brown-rot Fungi.
V. Brown-rot Blossom Wilt of Pear Trees.

PLATE XLI.

Fig. 1. Pear blossom wilt, summer condition (natural infection).

Fig. 2. Pear blossom wilt, winter condition, showing two infected spurs (natural infection).

Fig. 3. One of the spurs shown in Fig. 2, enlarged ($\times 2$) to show conidial fructifications of *Sclerotinia cinerea* on the withered flowers and leaves.

PLATE XLII.

Fig. 4. Natural infections on Fertility pear. Above is a dead spur infected the previous year, and below are two inflorescences recently infected.

Fig. 5. The branch on the left bears four infected inflorescences, the result of inoculation with conidia from a pure culture of a strain of *S. cinerea* isolated from a pear spur.

Fig. 6. A pear inflorescence showing an infected flower in the centre; this flower had been artificially inoculated with conidia of *S. cinerea*; note the blackened pedicel and the withered stamens and calyx lobes.

Fig. 7. A dead pear spur in March, 1925. Flowers of this spur had been artificially inoculated with *S. cinerea* in April, 1924.

Fig. 8. A pear tree bearing a number of withered inflorescences, each of which had been inoculated on two flowers with conidia of a cherry strain of *S. cinerea*.



WORMALD — BROWN ROT BLOSSOM WILT.

Huth coll.



4



5



8



6



7

WORMALD - BROWN ROT BLOSSOM WILT.

